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3M COMPANY

Recommendation Regarding

Prioritization of

PFOA

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EXECUTIVE SUMMARY

The Office of Environmental Health Hazard Assessment ("OEHHA") of the California Environmental Protection Agency is soliciting public comments on five chemicals proposed for the Developmental and Reproductive Toxicant Identification Committee ("DARTIC") consideration as candidates for potential listing as reproductive and developmental toxicants for purposes of Proposition 65. Among the five chemicals proposed in their August 2015 Notice, perfluorooctanoic acid ("PFOA") had 20 analytical epidemiologic studies considered as having "adequate quality reporting an association between exposure to the chemical and increased risk of adverse developmental or reproductive outcomes plus additional studies in humans and animals" and was recommended to be considered in the prioritization.

The purpose of Proposition 65 (the Act) is to protect Californians from exposure to reproductive toxicants (and carcinogens) through the discharge prohibition and warning requirement that the Act imposes. PFOA is a perfluoroalkyl carboxylate that 3M completed the phase-out of in 2008. 3M understands that the other major producers who are participants in the United States Environmental Protection Agency's PFOA Product Stewardship Program intend to complete their commitment as well by 2015. We respectfully submit that further review of PFOA is not necessary to accomplish the goals of the Act, and would unnecessarily divert the OEHHA's and DARTIC's valuable resources that otherwise could be invested in other efforts where more meaningful public benefit would result, for the following reasons:

1. ***Efforts that Restrict Manufacture, Import, and Use of PFOA and PFOA-Precursors in the United States.*** The production of PFOA in the United States will be completely ceased by major manufactures by the end of 2015 under a major product stewardship program implemented by the US Environmental Protection Agency (EPA). The EPA has and continues to promulgate Federal Regulations that substantially restricts the manufacture and use of PFOA and PFOA-precursors.

2. ***Declining Residues in Human Blood.*** There is an unmistakable downward trend in the levels of PFOA found in the U.S. general population in the last decade. Based on CDC's National Health and Nutrition Examination Survey (NHANES) data, mean blood levels of PFOA in the general population have declined by approximately 60% since 1999-2000.

3. ***Absence of Data That Would Support the Reproductive Toxicity.*** Our review of data identified in the Prioritization Notice, and other data that were omitted, are discussed in detail below.

   (i) The reported epidemiological associations between PFOA and reproductive toxicity in humans is likely confounded the underlying pharmacokinetics of PFOA; and

   (ii) Developmental observations reported in laboratory rodents for PFOA were primarily mediated by maternal effects (developmental effects in offspring associated with PFOA-

effected maternal animals). In addition, rodents may not the most appropriate for the hazard assessments of PFOA for the developmental toxicity in humans due to demonstrated differences in mode of action data.

4. ***An Ample Margin of Safety.*** Even if PFOA was a strong candidate for listing (which is not supported by the data), the levels of PFOA causing a potential in reproductive / developmental toxicity in mice are *three orders of magnitude* higher than the levels experienced by the general population, demonstrating an ample margin of safety.

In conclusion, the above four points lead to the reasonable conclusion that PFOA ***should not be assigned a high priority*** for review by OEHHA. In the details that we provide below, we address each of the above points, including the issues related to exposure and the human and animal data relevant to the potential reproductive toxicity of PFOA.

DISCUSSION

1. Efforts that Restrict Manufacture, Import, and Use of PFOA and PFOA-Precursors in the United States.

In May 2000, 3M announced that it was voluntarily phasing out the production of perfluorooctanyl chemistry, including PFOA. This goal was reached by 2008. In 2006, the US Environmental Protection Agency (EPA) invited eight major fluoropolymer and telomer manufacturers (Arkema, Asahi, BASF (successor to Ciba), Clariant, Daikin, 3M/Dyneon, DuPont, and Solvay Solexis) to join in a global stewardship program with two goals: 1) To commit to achieve, no later than 2010, a 95 percent reduction, measured from a year 2000 baseline, in both facility emissions to all media of PFOA, precursor chemicals that can break down to PFOA, and related higher homologue chemicals, and product content levels of these chemicals; and 2) To commit to working toward the elimination of these chemicals from emissions and products by 2015. In January 2015, EPA released the most recent reports that showed these companies were on track to reach the program's goal of phasing out these chemicals by the end of 2015. Annual progress reports can be found at http://www.epa.gov/oppt/pfoa/pubs/stewardship/preports8.html (accessed October 7, 2015).

EPA has and continues to promulgate Federal Regulations that substantially restricts the manufacture and use of PFOA and PFOA-precursors.

2. Residual Levels of PFOA in Blood in the United States General Population Have Declined by Sixty Percent since 1999-2000

The CDC's National Health and Nutrition Examination Survey, a nationally representative sample of the U.S. population (noninstitutionalized), has conducted biomonitoring of selected environmentally-present chemicals every 2 years since 1999-2000. This includes PFOA. The geometric mean concentration of PFOA in the serum (blood) of the general population has *declined by approximately sixty percent* since 1999-2000 (Figure 1A). The geometric mean concentration went from 5.41 ng/mL (1999-2000) to 2.08 ng/mL (2011-2012). The 95th percentile has declined from 11.9 ng/mL (1999-2000) to 5.68 ng/mL (2011-2012) (Figure 1B). This decline in PFOA was observed across both sexes (see Figures 1A and 1B), as well as for age (see Figures 2A and 2B), and ethnicity/race (see Figures 3A and 3B). Data obtained for these figures are found in the Fourth National Report on Human Exposure to Environmental Chemicals (http://www.cdc.gov/biomonitoring/pdf/FourthReport_UpdatedTables_Feb2015.pdf, accessed October 7, 2015).

Figure 1A. Trends in NHANES by Sex:
Geometric Mean Serum PFOA Concenrations (ng/mL)

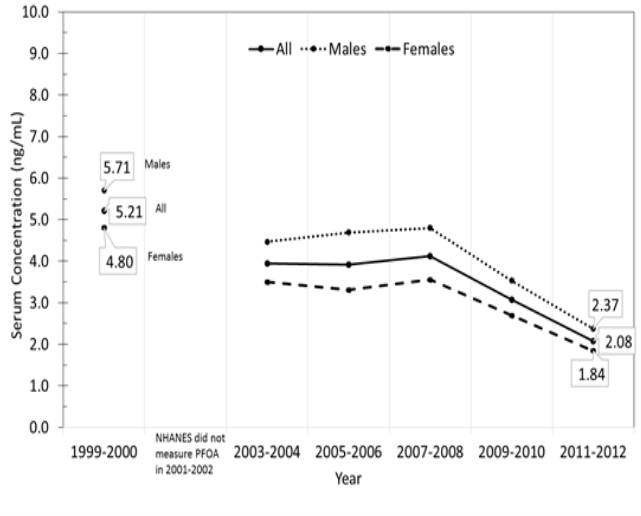


Figure 1B. Trends in NHANES by Sex:
95th Percentile Serum PFOA Concentrations (ng/mL)

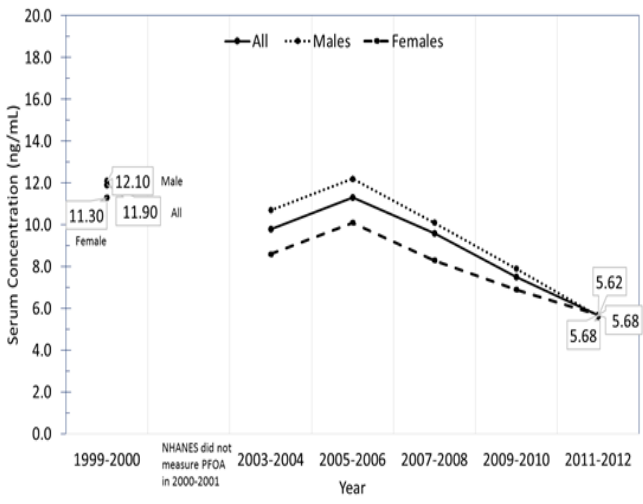


Figure 2A. Trends in NHANES by Age:
Geometric Mean Serum PFOA Concentrations (ng/mL)

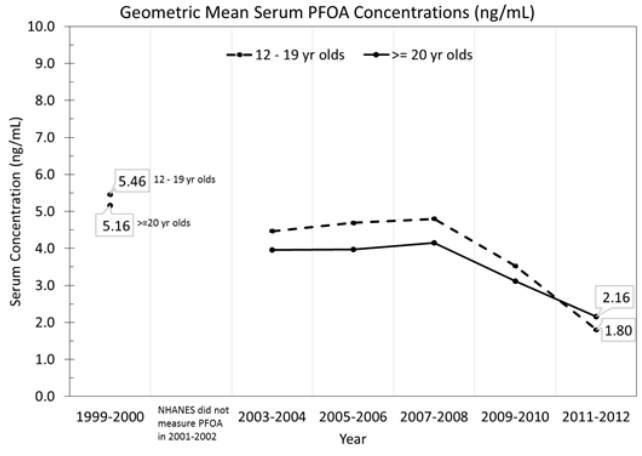


Figure 2B. Trends in NHANES by Age:
95th Percentile Serum PFOA Concentrations (ng/mL)

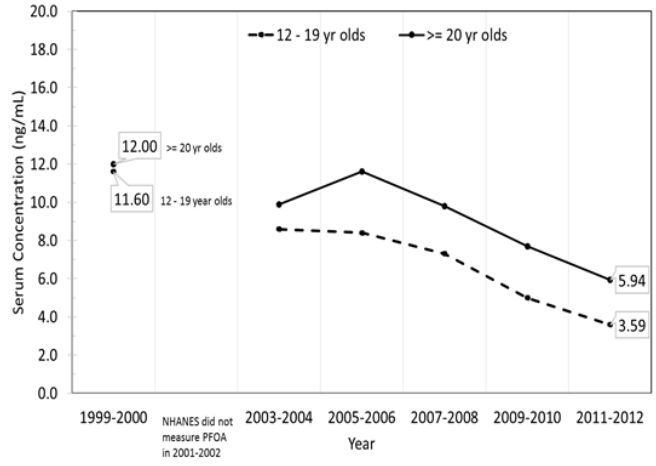


Figure 3A. Trends in NHANES by Ethnicity/Race:
Geometric Mean by Serum PFOA Concentration (ng/mL)

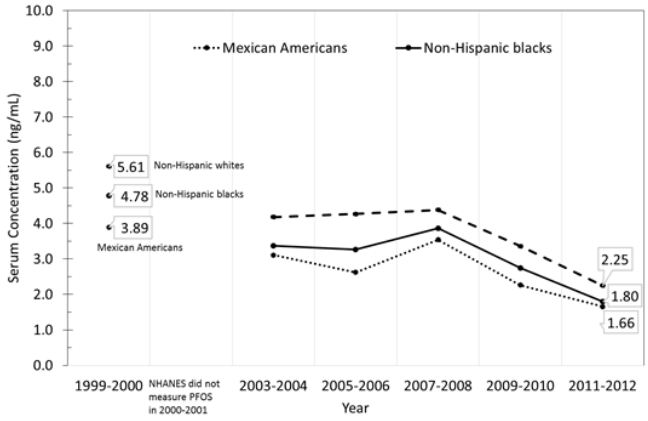
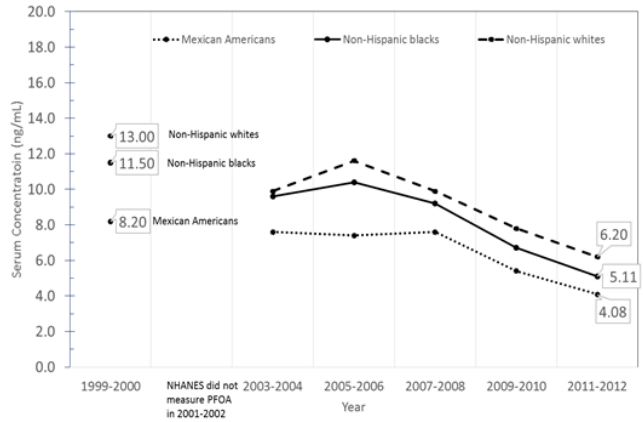


Figure 3B. Trends in NHANES by Ethnicity/Race:
95th Percentile Serum PFOA Concentrations (ng/mL)



Other cross-sectional biomonitoring studies, including analyses from six American Red Cross blood donation centers, between 1999-2000 and 2010, have shown similar declining trends in PFOA serum concentrations (Olsen et al. 2003; Olsen et al. 2012; Olsen et al. 2008). One of these blood donation centers is the American Red Cross Southern California Region located in Los Angeles. Biomonitoring California has presented geometric mean results of several studies that it has sponsored since 2010. The largest Biomonitoring California study, the California Teachers Study (CTS), reported a geometric mean serum PFOA concentration of 2.51 ng/mL and a 95th percentile of 6.27 ng/mL for serum samples collected between 2011-2013 from 856 primarily white women. These findings are comparable to the NHANES results for this time period (see figures above).

As a result of the EPA PFOA product stewardship program, it is anticipated that PFOA serum concentrations will continue to decline. Biomonitoring data for PFOA for the 2013-2014 period is anticipated to be released by NHANES within two years. The American Red Cross blood donor study is currently analyzing blood samples collected in July 2015 from the same six donation centers in their previous studies conducted in 2000, 2006, and 2010 (Olsen et al. 2003; Olsen et al. 2012; Olsen et al. 2008). Ongoing Biomonitoring California study findings for PFOA can be found on its website. (http://www.biomonitoring.ca.gov/sites/default/files/downloads/California_Teachers_Study_PFCs_07112013_1.pdf, accessed October 7, 2015)

3. Absence of Data That Would Support the Reproductive Toxicity

The Prioritization Process requires the OEHHA staff to screen chemicals for reproductive effects based on human epidemiological and laboratory experimental data. Although the prioritization process evaluates chemicals in a somewhat qualitative manner, the evaluation of studies against Proposition 65 listing criteria is a useful measure of how a chemical should be prioritized. In these comments we discuss numerous studies not identified in OEHHA's Prioritization Notice. We submit that current data would not support a listing decision. This conclusion, combined with the cessation of PFOA production and importation, as well as the steep decline in blood serum levels, warrant a finding that PFOA should not be designated as a high priority chemical.

A reproductive toxicant is defined by the State as follows:

- A chemical is deemed "known to the State to cause reproductive toxicity" if "it has been *clearly shown* through scientifically valid testing according to generally accepted principles to cause reproductive toxicity."[1]
- For definition purpose, "reproductive toxicity" includes developmental toxicity, female reproductive toxicity, and male reproductive toxicity and it is recommended that "a weight-of-evidence approach" be used when evaluating the available data.[2]
- According to OEHHA, it requires a "causal relationship between the chemical and reproductive toxicity in the human data."[3] Or, it requires "studies in experimental

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1 Cal. Health & Safety Code § 25249.8(b). (emphasis added)

2 http://oehha.ca.gov/prop65/policy_procedure/pdf_zip/dartCriteriaNov1993.pdf

3 Cal. Code Regs., *tit*.27, §25306(g)(1).

> animals indicate that there are sufficient data, taking into account the adequacy of the experimental design and other parameters such as, but not limited to, route of administration, frequency and duration of exposure, numbers of test animals, choice of species, choice of dosage levels, and consideration of maternal toxicity, indicating that an association between adverse reproductive effects in humans and the toxic agent in question is biologically plausible."[4]

In the section titled "**Human Data**" below, some relevant epidemiological studies that address human reproductive data are discussed and the weight-of-evidence does not support a causal relationship between PFOA and reproductive toxicity in humans. In the section titled "**Animal (mammalian) Data**" below, several comprehensive reproductive and developmental toxicity studies are also discussed. These studies provide strong evidence that many of the developmental outcomes reported in laboratory rodents are the consequence of maternal effects. In addition to the fact that these effects occurred at serum PFOA concentrations that are several orders of magnitude higher than general population, mode-of-action data further suggest that rodents may not be the most appropriate species for the hazard assessment of PFOA toxicity in humans.

Human Data:

Several associations that have been reported in the OEHHA epidemiological screen are likely confounded by the underlying pharmacokinetics of PFOA as related to the physiology and/or pathology of the outcomes studied.

Longnecker (2006) commented the advent of modern analytical chemistry not only enabled lower concentrations of environmental chemicals to be biomonitored but also allowed for a great proportion of the variation measured could be accounted for by differences in subjects' metabolism and excretion. Longnecker opined that the low concentrations measured may be a reflection of the byproduct of the underlying pharmacokinetics, systems biology, and pathogenesis. Several of the epidemiologic associations that have been identified as statistically significant findings in the OEHHA epidemiologic screen process may be confounded by the underlying pharmacokinetics of PFOA as related to the pathophysiology of these outcomes. This includes epidemiologic associations related to PFOA and time to pregnancy (subfecundity), birth weight, delayed menarche, decreased breast feeding duration, early onset menopause, and endometriosis.

As stated in the OEHHA criteria for recommending chemicals for listing, sufficient evidence in humans to list as "known to the state to cause reproductive toxicity" requires epidemiological studies to be scientifically valid according to generally accepted principles, provide convincing evidence to support a causal relationship between exposure and the developmental or reproductive effect in question which requires accurate exposure and toxicity endpoint classification *and proper control of confounding factors*, bias and effect modifiers (italics added).

We illustrate three examples that OEHHA will encounter as it ascertains whether there was "proper control of confounding factors." These examples are: a) PFOA and time to pregnancy; b) PFOA and birth weight; and c) PFOA and delayed onset to menarche.

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[4] Cal. Code Regs., *tit*.27, §25306(g)(2).

a). Time to Pregnancy

The initial investigation that suggested an association between PFOA and increased infertility and decreased fecundability was a study of the Danish National Birth Cohort (DNBC) (Fei et al. 2009). The DNBC was a nationwide follow-up study of approximately 100,000 children and their mothers. Pregnant women in their first trimester were recruited through their physicians. Fei et al. randomly selected 1400 women from all participants (n = 43,045) who gave birth to a single live born child without congenital malformation and who participated in a set of 4 telephone interviews, including questions regarding the length of time required to have achieved a planned successful pregnancy. Blood samples (weeks 4 – 14 of pregnancy) were used to measure PFOA among the 1240 women who met this definition. Infertility was defined as reporting a time to pregnancy (TTP) > 12 months or infertility treatments for this current pregnancy. Fecundity odds ratios (FORs) were calculated that measured the odds of a successful conception for women who had higher levels of PFOA compared with the reference level within a given calendar month, given that pregnancy was not achieved in the prior month. FORs < 1 indicate decreased fecundity and a longer TTP.

Among the 1240 women with planned pregnancy, their mean PFOA concentration was 5.6 ng/mL. The mean PFOA concentrations by time to pregnancy (number of participants in parentheses) were 5.4 ng/mL at < 6 months (n = 861), 6.0 ng/mL at 6 – 12 months, (n = 191), and 6.3 ng/mL at >12 months (n = 188).

Provided in Table 1 are the odds ratios for infertility and fecundability from the Fei et al. study (2009). These odds ratios were adjusted for maternal age at delivery, parity, pre-pregnancy BMI, maternal SES, alcohol consumption before pregnancy, paternal age, and paternal education. There were statistically significant trends for infertility and fecundability with PFOA. Fei et al. acknowledged that the exposure time window of interest was at the start of pregnancy planning but their exposure data for PFOA were measured at 4 – 14 weeks gestation and would have been rather stable over pregnancy due to the long elimination rate for PFOA in humans. Fei et al. suggested exposure to PFOA at levels found in the general population may increase TTP and could explain some of the fertility differences among different populations developed countries

Based on their review of the Fei et al. (2009) data, Olsen et al. (2009) discussed that parity is both an outcome of fecundity and is associated with perfluoroalkyl concentrations. Because perfluoroalkyl levels would be lower after a pregnancy, a longer interval between births would result in more time for a woman to absorb concentrations that could replace the loss incurred from the birth. In other words, there would be a longer time for reaccumulation to occur. Women who begin with comparable perfluoroalkyl concentrations and equal parity may have different perfluoroalkyl concentrations at their next birth based on the time elapsed between births (which includes the time required to become pregnant). Olsen et al. surmised if all else is equal, those women with longer TTP will have longer intervals of time between births and so may have higher perfluoroalkyl levels prior to the next pregnancy. This would result in an association between perfluoroalkyl concentrations and TTP but the direction of the causality would be backwards (*i.e.*, reverse causation).

Whitworth et al. (2012) elaborated upon this reverse causation hypothesis in a case-control study of women who originated from the Norwegian Mother and Child Cohort (MoBa) Study. Women were restricted to those who delivered a live-born child and provided a plasma sample around 17 weeks of gestation. Subfecund cases (n = 416) were defined as TTP > 12 months. Controls (n = 494) were defined as TTP ≤ 12 months. Median PFOA concentrations were 2 ng/mL for both subfecund cases and controls. Whitworth et al. stratified their results by parity (nulliparous vs. parous). Parity was not considered a potential confounder because it is influenced by a woman's underlying fecundability. Among parous women, the interval between the 2 most recent pregnancies, the number of previous pregnancies, and the duration of breast-feeding were examined for their influence on measured levels of PFOA.

Among parous women, Whitworth et al. (2012) reported odds ratios for TTP of similar magnitude as Fei et al. for PFOA (Table 1). However, among nulliparous women, they reported odds ratios for TTP below null and the trend appeared to decrease with increasing PFOA concentrations. Whitworth et al. concluded that due to the pharmacokinetics of perfluoroalkyls during pregnancy, delivery, and lactation, associations between PFOA and subfecundity may be produced when a causal association does not exist. They recommended studying nulliparous women regarding the potential reproductive toxicity of perfluoroalkyls.

Because PFOA was not measured at the beginning of the time to pregnancy interval but after a pregnancy had been achieved, Fei et al. (2012) acknowledged in a commentary that TTP could have potentially influenced the measurement of PFOA in their original data (Fei et al. 2009). Fei et al. (2012) then reanalyzed their data by stratifying on parity and concluded there was limited evidence for reverse causation as an explanation for their results. As shown in the Table 1, upon stratification by parity, Fei et al. (2012) found the odds ratios for infertility or fecundability attenuated to the null in the nulliparous women. This suggests reverse causation.

In a third analysis of the DNBC data, Bach et al. (2015) analyzed a second (new) participant subset of the DNBC that differed somewhat in methodology, including covariates, from the original study as published by Fei et al. (2009). In this second subset, there were 65% fewer subjects (n = 440) than the original study described above. Median PFOA serum concentration was slightly less (4.0 ng/mL). For PFOA, a similar association was observed by Bach et al. among parous but not nulliparous as shown by the first subset reanalysis by Fei et al. (2012), which was similar to the reanalysis of the first DNBC subset data reported by Fei et al. (2012). See Table 1 for the Bach et al. results. This continued to suggest a "reverse causation" argument.

Other studies have been published including relatively small prospective cohort studies by Vestergaard et al. (2012) and (Buck Louis et al. 2013). Neither have shown an association between TTP and PFOA. Nor have associations been reported between TTP and PFOA by Jørgensen et al. (2014).

Vélez et al. (Vélez et al. 2015) recently reported on a data set from the Canadian Maternal-Infant Research on Environmental Chemicals (MIREC) cohort. Information on TTP and maternal blood concentrations was collected during the first trimester of pregnancy (6 to < 14 weeks) of the current pregnancy. The median PFOA concentration was 1.7 ng/mL. A total of 1,625 subjects were included in this analysis. Concentrations were log-transformed and divided by their SDs. The adjusted odds ratio for infertility (TTP > 12 months or infertility treatment) for PFOA was 1.31 (Table 1). Adjusted fecundability odds ratio was 0.89. However, unlike all published studies before them (except Fei et al. 2009), Vélez et al. chose not to conduct analyses stratified by parity (nulliparous vs. parous) because, in their opinion, their hypothesized causal model suggested to do so would condition on a collider (the previous time to pregnancy which they considered to be a proxy for parity). Conditioning on parity would result in collider-stratification bias according to them. However, the Velez et al. model did not acknowledge that the timing of the measurements of PFOA occurs after the conception (not before) and therefore TTP may indeed influence PFOA measurements among parous women.

In summary, women with longer TTP will have longer intervals of time between given births and therefore may reaccumulate higher PFOA levels prior to the next pregnancy compared to women with shorter TTP. This would result in longer TTP measurements associated with higher PFOA levels, but the direction of the causality would be backwards; it would be the longer time between births (including the TTP) that resulted in higher PFOA concentrations.

b). Birth weight

A set of 4 papers was published in *Environmental Health Perspectives* in October 2014 that provided a "comprehensive and transparent assessment on the nonhuman mammalian and human evidence of whether fetal growth, in particular birth weight at term, was inversely associated with exposure to PFOA or its salts" (Johnson et al. 2014; Koustas et al. 2014; Lam et al. 2014; Woodruff and Sutton 2014). These investigators used the Navigation Guide methodology to conduct a rigorous approach to research synthesis that has been developed to reduce bias and maximize transparency in the evaluation of environmental health information (Woodruff and Sutton 2014). The evaluation process involved three steps: 1) specify the study question; 2) select the evidence; and 3) rate the quality and strength of the evidence according to consistent criteria, and performing appropriate statistical analyses (*e.g.*, meta-analyses). For each systematic review of the nonhuman mammalian and human data, the strength of evidence was defined as either sufficient, limited, inadequate, or lack of evidence of toxicity. Integration of each separate rating for nonhuman and human data resulted in an overall final strength of evidence rating.

Koustas et al. (2014) addressed the question of whether PFOA or its salts affected fetal growth in animals. They initially reviewed 21 toxicology studies relevant to the question. They determined only a subset of the data, 8 mouse gavage data sets from 7 studies, could be combined for their meta-analysis of birth weight in relation to PFOA doses administered. Only the low PFOA doses were considered in their meta-analysis in order to minimize adverse impacts from higher administered doses in these studies. The

mouse species was chosen, as compared to the rat, due to its longer half-life of PFOA and pharmacokinetic differences between the sexes. The meta-analysis estimate of PFOA calculated form these 8 data sets was a change in mean pup birth weight of -0.023 g (95% CI -0.029, 0.016) per 1-unit increase in dose (mg/kg body weight per day). Koustas et al. (2014) summarized the strength of evidence across the 8 mouse gavage data sets as "sufficient evidence of toxicity" based on their a priori definition of 'one or more well-designed, well-conducted studies' and the conclusion is unlikely to be strongly affected by findings from future studies.

Similarly, Johnson et al. (2014) reported the systematic review of the human evidence by identifying 18 epidemiologic studies of which 9 data sets were considered combinable in a meta-analysis for birth weight and PFOA exposure. These 9 data sets represented 4,149 births. Reviewing each study for risk of bias (recruitment strategy. blinding, exposure assessment, confounding, incomplete outcome data, selective outcome reporting, conflict of interest, and other bias), Johnson et al. rated the quality of evidence across the studies as 'moderate.' Their meta-analysis of these 9 data sets reported an estimate of -18.9 grams (95% CI -29.8, -7.9) birth weight per ng/mL increase in serum or plasma PFOA (see Figure 4). Johnson et al. summarized the strength of human evidence as 'sufficient evidence of toxicity' based on a reduction in birth weight associated with PFOA exposure and that chance, bias and confounding were ruled out with reasonable confidence.

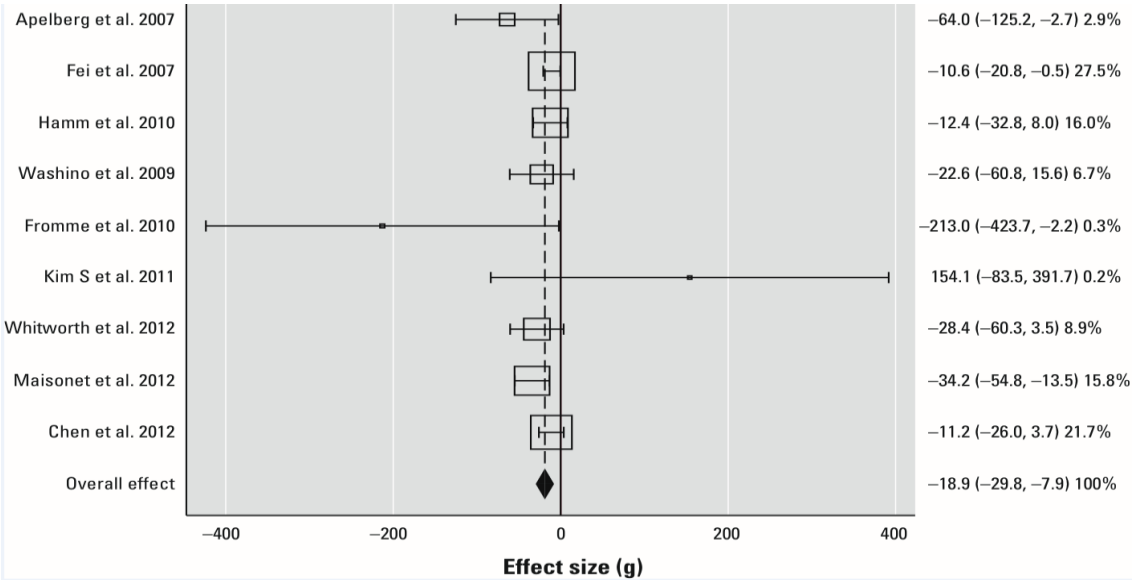

Figure 4 – from Johnson et al. 2014 Environ Health Perspect 122 1028-1039
Results of meta-analysis for birth weight (n = 9 studies, 4,149 births) shown as effect estimates [change in birth weight in grams per nanogram of PFOA per milliliter of serum or plasma (95% CIs)]. The percentages are weightings of the individual studies in the meta-analysis according to the inverse of the variance, and the sizes of the boxes are scaled accordingly. The dashed line indicates the overall effect estimate derived from the meta-analysis, and the diamond indicates the 95% CI of the overall effect estimate.

Lam et al. (2014) (same group of authors as Johnson et al. and Koustas et al.) subsequently integrated the strength of the nonhuman mammalian and human ratings and reached the conclusion that PFOA is 'known to be toxic' to human reproduction and development based on sufficient evidence of decreased fetal growth in both nonhuman mammalian and human species.

However, none of 9 epidemiologic studies included in the meta-analysis by Johnson et al. (2014) considered the potential confounding that could arise from the glomerular filtration rate (GFR). The maternal GFR increases within one month of conception (Helal et al. 2012) with maternal GFR and renal blood flow increasing by 40 – 65% and 50 - 85%, respectively, during a normal pregnancy. Whitworth et al. (2012) suggested that, because GFR is diminished in lower weight infants, this could lead to less renal elimination of PFOA; thus raising the question whether the epidemiologic studies that assessed a relationship between birth weight and PFOA were confounded by not adjusting for GFR.

While acknowledging the above hypothesis by Whitworth et al. (2012), Lam et al. (2014) believed their overall conclusion was not undermined for two reasons: 1) it was not relevant to the nonhuman mammalian data; and 2) their systematic review of the literature for this relationship between birth weight and maternal glomerular filtration rate did not suggest sufficient evidence to support this hypothesis. However, unlike their meta-analysis on PFOA and birth weight (Johnson et al. 2014), Lam et al. did not provide a systematic review in their paper as to how they reached their conclusion of a lack of an association between GFR and birth weight. Such a review by this set of authors was published a few months later by Vesterinen et al. (2015)

In their review, Vesterinen et al. (2015) presented three relationships to consider in assessing fetal growth: 1) fetal growth and GFR; 2) fetal growth and plasma volume expansion (PVE); and 3) PVE and GFR. (See Figure 1 in the Supplement to the Vesterinen et al. paper.) They examined 35 studies through the same Navigation Guide methodology. Vesterinen et al. found consistent evidence of an association among studies reporting the relationship between birth weight and PVE but they found the studies between GFR and birth weight were inconsistent and the majority had small sample sizes (range 9 to 283). They also had low confidence in the studies that examined the relationship between PVE and GFR. Vesterinen et al. concluded "the strength of the evidence of an association between fetal growth and GFR was not classifiable based on the low quality and indeterminate direction of effect of human studies and the small number and size of non-human mammalian studies which were of low quality with indeterminate direction of effect." Nevertheless, Vesterinen et al. acknowledged "A well-conducted observational human study could increase our confidence in the strength of the association" and because "the review process involved judgments, a different group of researchers *at a different time* might reach a different conclusion." (italics added).

Several months later, Verner et al. (2015), who had the distinct advantage of having one additional critically-important paper (Morken et al. 2014) not available to Lam et al. (*i.e.*, not yet published), concluded "there is reason to believe a true association

exists between maternal GFR during pregnancy and birth weight." Morken et al. examined a sub-cohort of 953 women (470 women with and 483 women without preeclampsia) in the Norwegian Mother and Child Cohort (MoBa) study. The sample size represented 29 more subjects than the combined total (n = 924) from the 13 small sample studies that were available to Lam et al. at the time of their review (as discussed above). Morken et al. found a statistically significant association between maternal GFR in the second trimester and infant birth weight with using two different GFR formulas in the total cohort, but not with a third estimated GFR formula. The inclusion of women with preeclampsia in this study increased the study power because it increased the proportion of small-for-gestational age infants in the analysis. In a different analysis, Morken et al. analyzed the 953 women in a model of birth weight in relationship to the concentration of PFOA that was measured in these subjects' serum. Adjustment for GFR attenuated the PFOA coefficient by 66%.

Upon their review of the literature, and concluding there was likely a true association between maternal GFR and birth weight, Verner et al. (2015) modified an existing physiologically based pharmacokinetic model (PBPK) of pregnancy and lactation and PFOA (Loccisano et al. 2012; Loccisano et al. 2013) to address how much of the PFOA and birth weight association might be attributable to GFR. They compared simulated estimates from their PBPK model to those from their meta-analysis of 7 epidemiologic studies (all included in the meta-analysis by Johnson et al.) See Figure 5 below.

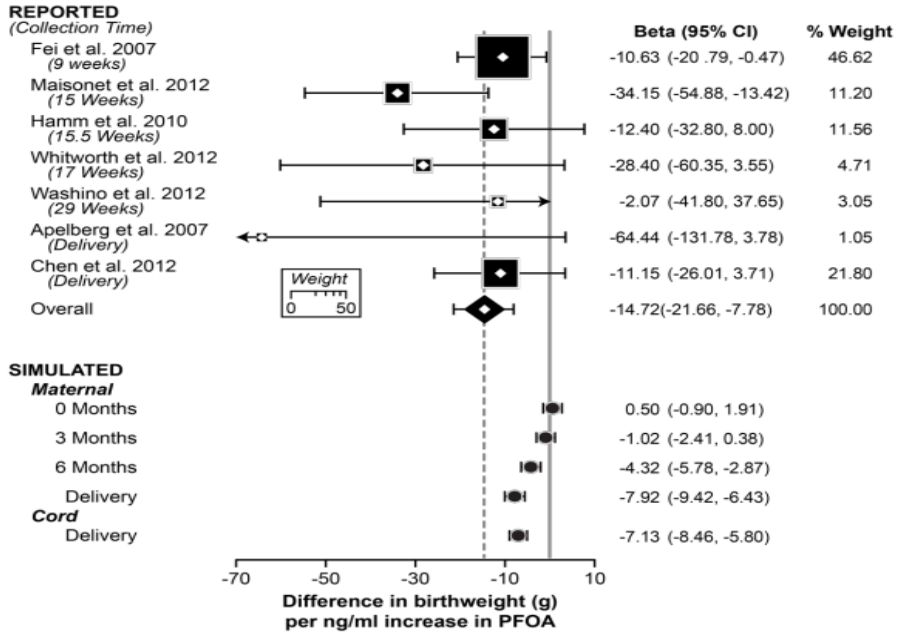

Figure 5 – from Verner et al. 2015, Environ Health Perspect DOI: 10.1289/ehp.1408837. Difference in birth weight (g) per 1 ng/mL increase in Reported (meta-analysis) and Simulated Model PFOA levels. The Simulated Model showed a -7.92 g birth weight per ng/mL maternal PFOA and -7.13 g birth weight per ng/mL cord plasma PFOA. The size of the square represents the weight of each study in the calculation of the overall meta-analytic association.

Using Monte Carlo and sensitivity analyses, Verner et al. reported the association between maternal plasma levels (per 1 ng/mL increase) and birth weight only appeared after the first trimester (see Figure 5, Simulated Model). The association was strongest at birth. The association between simulated PFOA levels (per 1 ng/mL increase) and birth weight was comparable for maternal plasma at term (-7.9 g birth weight (95% CI -9.4, -6.4)) and cord plasma (-7.1 g birth weight (95% CI -8.5, -5.8)). Their meta-analysis of the 7 epidemiologic studies (see Figure 5, Reported Model) found a summary meta-analysis coefficient of -14.7 g (95% CI -21.7, -7.8) birth weight for each 1 ng/mL increase of PFOA (as compared to the -18.8 g for 1 ng/mL increase in PFOA as reported by Johnson et al. as illustrated in Figure 4). Verner et al. concluded a substantial proportion of the association between maternal PFOA and birth weight may be attributable to confounding by GFR. Also, Verner et al. concluded epidemiologic studies that measured PFOA early in pregnancy may have been less confounded by GFR than those who measured PFOA late in pregnancy.

In summary, epidemiological associations between maternal PFOA and birth weight are confounded by GFR.

c). Delayed menarche

Based on the cross-sectional C8 Health Project data obtained in 2005-2006, Lopez-Espinosa et al. (2011) categorized 3,067 boys and 2,931 girls aged 8 – 18 years as whether they had reached puberty based on sex steroid hormone levels or onset of menarche. Using total testosterone (> 50 ng/dL) or free testosterone (> 5 ng/dL) in boys and self-reported menarche and estradiol >20 pg/mL in girls as markers of puberty, PFOA (girls only) was associated with median delays of three to six months based on quartile analyses. The authors acknowledged that clearance may have an explanatory role, as an earlier menarche would result in behavioral and physiologic changes, including menstrual blood loss, which may result in lower perfluoroalkyl levels. The delayed menarche association reported by Lopez-Espinosa et al. was inconsistently reported in two longitudinal studies (Christensen et al. 2011; Kristensen et al. 2013). Christensen et al. (2011) conducted a nested case-control study within a cohort of approximately 14,000 pregnant women in 1991-1992. Cases were defined as female offspring who self-reported early menarche before 11.5 years (n = 218) with a median PFOA concentration of 3.9 ng/mL compared to 3.6 ng/mL amongst the controls (menarche after 11.5 years (n = 230)). The adjusted odds ratios for a natural log transformed unit of PFOA was 1.01 (95% CI 0.61 – 1.68) for an age at menarche having occurred at less than 11.5 years of age. Kristensen et al. (2013) examined the recalled age of menarche among 343 daughters aged 20 years whose mothers had an archived blood sample measured while at pregnancy week 30. Mean age at menarche was 13.2 years, median maternal PFOA was 3.6 ng/mL. Daughters exposed to PFOA *in utero* had a 5.3 months (95% CI 1.3 – 9.3) later age of self-reported menarche among the highest exposed group (maternal PFOA level 4.4 – 19.8 ng/mL) compared to the referent group (0.1 – 3.0 ng/mL PFOA maternal level).

A Monte Carlo PBPK simulation model was developed that incorporated significant points of pubertal development that included growth spurts and menarche (Wu et al. 2015). The model included compartments for plasma, gut, liver, fat, rest of body, kidney, filtrate, and storage. Tissue volumes and tissue blood flow rates were estimated based on body

weight, body height, body surface area, and body mass index. Daily exposure to PFOA in plasma was from several sources but it was only drinking water and absorbed into gut for PFOA (per the mid-Ohio river population studied by Lopes-Espinosa et al. 2011). PFOA concentrations were simulated for a distribution of individuals 2 to 20 years of age with similar physiologic characteristics as those reported by Lopez-Espinosa et al. Models of growth were based on simulated population matches of the 5^{th}, 50^{th}, and 95^{th} percentiles of the NHANES 2003-2004 data. Monte Carlo simulations showed the distribution of serum PFOA concentrations to be very similar between the PBPK model and the Lopez-Espinosa et al. study population. The delay in menarche in days per natural log of PFOA was approximately one-third that reported in the Lopez-Espinosa et al. paper (Table 2).

In summary, the association between serum PFOA concentrations and delayed age at menarche may be due, in part, to dilution (through growth of adolescents) and excretion (via menstruation).

Human Data Summary: This review of three epidemiological associations with PFOA (time to pregnancy, birth weight, and delayed menarche) demonstrates the confounding of the underlying pharmacokinetics of PFOA as related to the pathophysiology of these outcomes studied. The above three examples illustrate the challenges of interpreting the existing epidemiology literature. Several other epidemiologic associations (e.g., decreased breast feeding duration, early onset menopause, and endometriosis) that have been identified as statistically significant findings in the OEHHA epidemiologic screen process are also confounded by the underlying pharmacokinetics of PFOA as related to the pathophysiology of these outcomes. Whether confounding factors, bias, and effect modifiers have been *properly controlled* in these epidemiologic associations, as well as others, is a critical component of a proper evaluation of these studies for prioritization.

Animal (mammalian) Data:

A number of experimental animal (mammalian) toxicological studies on the reproductive and developmental effects of PFOA have been published (Abbott et al. 2007; Albrecht et al. 2013; Butenhoff et al. 2004; Gortner 1981, 1982; Lau et al. 2006; Staples et al. 1984; Yahia et al. 2010). These studies included detailed information on the reproductive and developmental toxicity with these compounds as well as valuable insights on the role of maternal effects and its attribution to the developmental outcomes in laboratory animals. Comprehensive review on the developmental toxicity of PFOA was first reported in 2004 (Kennedy et al. 2004; Lau et al. 2004) and updated subsequently (Abbott 2015; Andersen et al. 2008; Lau 2012; Lau et al. 2007).

Overall, PFOA did not affect male or female reproductive functions in the laboratory animals. These included estrous cycles, sperm parameters, mating index, fertility index, and reproductive organ morphology. The potential of PFOA to influence reproductive performance has been evaluated in mice, rats, and rabbits. Gestational exposure to ammonium PFOA did not affect the number of uterine implantation sites in various strains of mice such as CD-1, Sv129, PPARα knockout, and humanized PPARα (Abbott et al. 2007; Albrecht et al. 2013; Lau et al. 2006; White et al. 2007). At inhalation dose up to 25 mg/m^3/day of ammonium PFOA or oral doses up to 100 mg/kg/day given during gestation to rats did not affect mating, pregnancy, and implantation (Staples et al. 1984). Oral administration of ammonium PFOA up to 150 mg/kg/day in rats or 50 mg/kg/day

in rabbits during GD 6 – 15 (period of organogenesis) also caused reduced body-weight gain, however, they did not affect the ovaries or the reproductive contents of the dams (Gortner 1981, 1982). In a two-generation reproduction/developmental study in rats (Butenhoff et al. 2004), the reproductive outcome was not affected with at oral ammonium PFOA administration up to 30 mg/kg/day (the highest dose used in the study). There were no effects on the mating or fertility indices in either male or female rats. Male rats had normal sperm parameters (count, motility, morphology) and female rats had regular estrous cycling with normal gestation lengths, and microscopic examination did not reveal any abnormalities in sex organs. Furthermore, effects of PFOA on reproductive organ morphologies in male non-human primates were evaluated from a six-month oral study and results indicated no abnormalities (Butenhoff et al. 2002).

The developmental effects reported in the laboratory animals for PFOA were primarily mediated by maternal effects. In fact, experimental evidence demonstrates that developmental effects associated with PFOA exposures in offspring are observed only where there were significant effects in the maternal animals. Evidence involving maternal effects in the outcome of the developmental toxicity, as seen in the disruption of maternal homeostasis, include the following examples.

Using the mouse developmental study data reported by Lau et al. (2006), which was the critical study chosen by U.S. EPA Office of Water for the derivaiton of the Provisional Health Advisory for PFOA issued in 2009 (USEPA 2009), there were statistically significant ($p \leq 0.05$), dose-related increases in maternal liver weight observed at doses 1 mg/kg/day ammonium PFOA or higher (the corresponding serum PFOA concentration was 21,900 ng/mL at the end of gestaton). Various develpmental effects were reported (*e.g.*, decrased postnatal survival, decreased body weight at birth and body-weight gain thereafter, and delays in eye openings) and they were only for litters from dams receiving 3 mg/kg/day or higher. Maternal responses clearly were present at doses that affected the fetus/neonate. In addition, because the influence of body weight on sexual maturation is well-described in the literature, it is not surprising that Lau et al. noted altered pubertal maturations in the offspring.

The developmental toxicity of ammonium PFOA has also been studied in rats (Butenhoff et al. 2004; Gortner 1981; Staples et al. 1984) and rabbits (Gortner 1982). In these studies, no increase in malformations relative to controls was observed at oral doses up 150 mg/kg/day in rats and 50 mg/kg/day in rabbits, as well as inhalation concentrations up to 25 mg/m^3/day (6 hours/day). In the studies by Gortner and by Staples et al., any effects on fetal or pup body weight were present at dose levels equivalent to or higher than those causing effects such as body weight in the maternal animals. In a two-generation reproduction/developmental study in rats (Butenhoff et al. 2004), F1-generation pups from the highest dose group (30 mg/kg) had decreased birth weight and reduced viability that were in apparent relationship to the corresponding reduced body weight at birth and weaning. These latter effects are similar to those observed in mice by others (Abbott et al. 2007; Lau et al. 2006; Yahia et al. 2010). Even though similar to observation by Lau et al. (2006) in that sexual maturation were slightly delayed (at the highest dose group only), there was no significant difference in F1 pups when days to sexual maturation was adjusted by (reduced) body weight.

In the recent years, there have been numerous studies that investigated the effects of PFOA on the developing mammary glands in mice as a consequence of exposure during either the *in utero*

or postnatal/peripubertal window (Albrecht et al. 2013; Macon et al. 2011; Tucker et al. 2015; White et al. 2007; White et al. 2009; White et al. 2011b; Yang et al. 2009; Zhao et al. 2010). Taken together, these studies demonstrate that the effects of PFOA on mammary gland development cannot be consistently described and quantified in mouse models because these studies either found *no effect, inhibition, or stimulation* of mammary gland development (see Table 3). Furthermore, the nursing capabilities of the dams from these studies did not appear to be affected despite altered the mammary gland developments. Therefore, even though there are data available on the mammary gland development in mice, a lack of concordance among all the studies brings into question the biological significance of this phenotype and its relevance to human health.

There has also been an increase in toxicological studies reporting on the endocrine disturbance potential with PFOA exposures. Most of these studies were done either under *in vitro* conditions (to which high concentrations of PFOA were employed) or *in vivo* but only with a limited set of endpoints evaluated such as selected gene expressions (D'Orazio et al. 2014; Dankers et al. 2013; Dixon et al. 2012; Du et al. 2012; Du et al. 2013; Gao et al. 2013; Kraugerud et al. 2011; Sales et al. 2013; Sonthithai et al. 2015; Wens et al. 2013; White et al. 2011a). Endocrine is a very complicated system and evaluation of endocrine functions is a very highly specialized field (this is especially true in human clinical medicine). Given that PFOA is a strong surfactant, the toxicity effects reported from the typical mono-layered *in vitro* tissue culture system offered very little insight and scientific value because the data were often comprised by the surfactant-induced toxicity. Similarly, gene expressions do not represent functionality and endocrine function is an intricate network.

Based on data from the large scale 2-generation reproductive and developmental studies (which are considered as the most comprehensive test by various agencies for evaluating endocrine functions), PFOA clearly did not alter the reproductive functions as the reproductive performances in both males and females were normal (*vide supra*). If PFOA were indeed an endocrine disrupting compound, then one would expect it to directly activate endocrine receptors such as estrogen receptors or thyroid receptors. Ishibashi et al. (2007) reported that PFOA did not activate human estrogen receptor α or β. Neither did Yao et al. (2014) report that PFOA can activate mouse or human estrogen receptors. Yao et al. also showed a lack of change in the histomorphology of uterine/cervix and vaginal tissues in female mice after receiving oral ammonium PFOA treatments. Furthermore, while triiodothyronine (T3, the active form of thyroid hormone) elicits a dose-response activation of human thyroid receptor α from 0.000001 – 0.01 uM, under the same study condition, there was no activation of human thyroid receptor α when exposed to ammonium PFOA up to 100 uM (3M Company, unpublished data).

The listing process also requires OEHHA staff to consider "sufficient evidence in experimental animals (mammals), such that extrapolation to humans is appropriate."[5] PFOA is a known activator for xenosensor nuclear receptors such as PPARα, constitutive androstane receptor (CAR), and pregnane X receptor (PXR) (Elcombe et al. 2010; Klaunig et al. 2003). It is well-documented that PFOA causes heptomegaly in rodents as a result of PPARα activation with some contribution from CAR and PXR. It is well-known that human liver is less responsive to the pleiotrophic effects of activation of PPARα or CAR (Gonzalez and Shah 2008; Klaunig et al. 2003; Lake 2009; Ross et al. 2010). Thus, with respect to PPARα and CAR-mediated effects in the liver

[5] http://oehha.ca.gov/prop65/policy_procedure/pdf_zip/dartCriteriaNov1993.pdf

and related metabolism, the human response is either attenuated or absent as compared to that of the rodents. Mechanistic studies have demonstrated that many of the observed effects upon PFOA exposure, including those observed in developing mice, can be explained, in part, by the activation of PPARα. Many of the developmental effects were either absent or attenuated when PFOA was administrated to PPARα knockout mouse. The influence of PPARα on the fetal developmental effects of PFOA in the Sv/129 mouse strain (wide-type vs. PPARα knockout) were investigated by Abbott et al. (2007) and Albrecht et al. (2013). While it is not possible to rule out completely the contribution of other modes of action(s), many of the developmental effects with PFOA described above were attenuated and/or improved with PPARα knockout mice such as post-natal survival and body weight effects. Given that rodents are more responsive and susceptible than humans to PPARα-mediated biological effects (*vide supra*) and PPARα may not play a critical role in normal development (Braissant et al. 1996; Lee et al. 1995); it brings into question the relevance of nuclear receptor-mediated effects in rodents and biological significance to humans

Animal (mammalian) Data Summary: The developmental effects reported in the laboratory animals for PFOA were primarily mediated by maternal effects and based on the recent mode of action data, rodents may not be the most appropriate species for the hazard assessment of PFOA on developmental toxicity in humans.

4. Even If PFOA Was A Strong Candidate for Listing (which is not supported by the data), the Margins of Safety Are Large Enough That It Should Not Be Assigned A High Priority for Review By OEHHA.

For all of the reasons articulated above, we believe that PFOA should not be considered for high prioritization for review as a reproductive toxicant by OEHHA. Moreover, the steady decline of PFOA serum concentrations in the United States general population (independently documented by CDC and 3M) is a reflection of effective risk management steps taken by US EPA companies like 3M to eliminate production and restrict almost all use, thereby greatly reducing exposures.

The establishment of margins of exposure (margins of safety) may also be informative in setting priorities. We can identify the margin of exposure between the serum PFOA concentrations to which people are exposed [low parts per billion] and the serum concentration in laboratory animals associated with the no effect level for developmental effects. Because the comparison is based on measured serum concentrations, it already accounts for species differences in toxicokinetics.

The reproductive / developmental study in mice by Lau et al. (2006) has been deemed as a critical study by US EPA Office of Water in setting the Provisional Health Advisory (2009) and a $BMDL_{10}$ of 0.46 mg/kg/day for maternal effects noted at term was derived. Because the lowest maternal dose used in that study was 1 mg/kg/day and the corresponding serum PFOA concentration was 21,900 ng/mL at term, maternal serum concentration at 0.46 mg/kg/day can be extrapolated to approximate 10,120 ng/mL.

Based on the 2012 NHANES data, compared to the geometric mean and 95% percentile serum PFOA concentration in the United States (2.08 ng/mL and 5.68 ng/mL, respectively), the

levels of PFOA causing a potential in reproductive / developmental toxicity in mice are *three orders of magnitude* higher than the levels experienced by the general population, demonstrating a large margin of safety.

Margin of Exposure for Human Exposure Compared to Benchmark Dose (Lower 95% Confidence Limit) for Reproductive / Developmental Effects in Mice		
Human Exposure Level U.S. General Population 2012 Data (NHANES)	**$BMDL_{10}$ In Mice**	**Margin of Exposure (Human Exposure Compared to $BMDL_{10}$ in Mice)**
Mean Serum PFOA Concentration (2.08 ng/mL)	10,120 ng/mL	4865
95^{th} Percentile Serum PFOA Concentration (5.68 ng/mL)	10,120 ng/mL	1782

For this reason, as well as the others above, PFOA ***should not be assigned a high priority*** for review by OEHHA.

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Table 1. Association between PFOA Plasma Concentrations (ng/mL) and Subfecundity Among 910 Subjects (416 Cases, 494 Controls) Subjects from the Norwegian Mother and Child Cohort Study, Norway, 2003-2004 (Whitworth et al. 2004)

		Infertility (> 12 months TTP)			Fecundability		
		Fei et al. (2009)	Fei et al. (2012)		Fei et al. (2009)	Fei et al. (2012)	
Fei et al. 2009; Fei et al. 2012		Adjusted OR (95% CI)			Adjusted OR (95% CI)		
	PFOA (ng/mL)	All Subjects	Nulliparous	Parous	All Subjects	Nulliparous	Parous
	< LLOQ – 3.91	1.00	1.00	1.00	1.00	1.00	1.00
	3.91 – 5.20	2.06 (1.22 – 3.51)	0.79 (0.30 – 2.08)	3.39 (1.75 - 6.53)	0.72 (0.57 - 0.90)	0.98 (0.59 – 1.64)	0.61 (0.46 – 0.80)
	5.21 – 6.96	2.54 (1.47 - 4.39)	0.55 (0.21 – 1.43)	2.92 (1.44 – 5.93)	0.73 (0.58 – 0.92)	0.93 (0.56 – 1.54)	0.62 (0.46 – 0.83)
	≥ 6.97	2.54 (1.47 – 4.39)	1.30 (0.52 – 3.21)	2.99 (1.28 – 6.98)	0.60 (0.47 – 0.76)	0.63 (0.39 – 1.04)	0.63 (0.44 – 0.91)
		P = 0.006	P = 0.082	P = 0.01	P ≤ 0.001	P = 0.002	P = 0.004
Whitworth et al. 2012		Adjusted OR (95% CI)					
	PFOA (ng/mL)	All Subjects	Nulliparous	Parous			
	< 1.66	1.00	1.00	1.00			
	1.66 – 2.24	1.6 (1.1 – 2.3)	0.6 (0.3 – 1.5)	1.5 (0.9 – 2.5)			
	2.25 – 3.02	2.5 (1.5 – 3.2)	0.6 (0.3 – 1.4)	2.4 (1.4 – 4.1)			
	≥ 3.03	2.0 (1.4 – 3.0)	0.5 (0.2 – 1.2)	2.1 (1.0 – 4.4)			
	Test for trend	P ≤ 0.001	P = 0.20	P = 0.01			
Bach et al. 2015		Adjusted OR (95% CI)					
	PFOA (ng/mL)	All Subjects	Nulliparous	Parous			
	< 3.0	1.00	1.00	1.00			
	3.0 - ≤ 5.2	0.92 (0.69 – 1.22)	0.82 (0.53 – 1.26)	1.30 (0.86 – 1.98)			
	> 5.2 - ≤ 5.5	0.94 (0.71 – 1.26)	1.11 (0.73 – 1.69)	0.96 (0.66 – 1.41)			
	> 5.5	0.86 (0.63 – 1.19)	0.99 (0.64 - 1.54)	0.74 (0.48 – 1.13)			
Vestergaard et al. 2012			All Subjects				
	PFOA (ng/mL)		Adjusted OR (95% CI)		Adjusted FOR (95% CI)		
	< 5.60		1.00		1.00		
	≥ 5.60		1.21 (.67 – 2.18)		0.92 (0.65 – 1.31)		
	Log-transformed (continuous)				1.18 (0.78 – 1.78)		
Buck Louis et al. 2013					All Subjects Adjusted FOR (95% CI)		
	PFOA Log-transformed and rescaled by the SD				0.95 (0.82 – 1.11)		
Velez et al. 2014			All Subjects				
			Adjusted OR (95% CI)		Adjusted FOR (95% CI)		
	PFOA Log-transformed and rescaled by the SD		1.31 (1.11 – 1.53)		0.89 (0.83 – 0.94)		
			P = 0.001		P < 0.001		

Table 2. Comparison of association between plasma PFOA concentrations and age at menarche in simulated (Wu et al. 2014) and observed (Lopez-Espinosa et al. 2011) girls.

Exposure	Simulated Wu et al. study			Lopez-Espinosa study		
	OR	95% CI	Delay (days)	OR	95% CI	Delay (days)
PFOA-Q2	0.95	0.88 – 1.02	12	0.54	0.35 – 0.84	142
PFOA-Q3	0.91	0.84 – 0.98	18	0.5	0l32 – 0.77	163
PFOA-Q4	0.82	0.76 – 0.88	48	0.57	0.38 – 0.89	130
LnPFOA	0.94	0.92 – 0.96	15	0.83	0.83 – 0.95	42

Table 3: Summary of mouse mammary gland findings

Authors	Species (strain)	Mammary Gland Outcomes
White et al. 2007	CD-1	Stunted
White et al. 2009	CD-1	Delayed
Yang et al. 2009	C57BL6	Stimulatory (5 mg/kg) Inhibitory (10 mg/kg)
Yang et al. 2009	Balb/c	Inhibitory
	C57BL/6	Stimulated
Zhao et al. 2010	CD-1	Delayed
Macon et al. 2011	CD-1	Delayed
White et al. 2011	CD-1	Delayed
Albrecht et al. 2013	Sv/129 WT	No effect
	PPARα KO	No effect
	hPPARα	No effect
Tucker et al. 2014	CD-1	Delayed
	C57BL/6	Delayed